Quantitative Trait Loci and Epistasis for Crown Freezing Tolerance in the 'Kanota' × 'Ogle' Hexaploid Oat Mapping Population

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ABSTRACT

Crown freezing tolerance is the most important factor conferring oat (*Avena* spp.) winter hardiness. The objective of this study was to identify quantitative trait loci (QTL) for crown freezing tolerance in the 'Kanota' × 'Ogle' recombinant inbred line (RIL) mapping population and to examine their relationship with other winter hardiness traits. One hundred thirty-five RILs were evaluated for crown freezing tolerance in a controlled environment. Previously published molecular marker and linkage map information was used for QTL detection. Seven QTL and four complementary epistatic interactions were identified that accounted for 56% of the phenotypic variation. Ogle contributed alleles for increased crown freezing tolerance at three loci, while Kanota contributed alleles for increased crown freezing tolerance at four loci. All loci where Kanota alleles increased crown freezing tolerance showed complementary epistasis for decreased crown freezing tolerance with the QTL near UMN13. Two of the major QTL identified were in the linkage groups (LG) associated with a reciprocal translocation between chromosomes 7C and 17, which was previously associated with spring growth habit in oat. The results confirm the importance of the chromosomes involved in the reciprocal 7C-17 translocation in controlling winter hardiness component traits.

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Abbreviations: LG, linkage group; MIM, multiple interval mapping; QTL, quantitative trait loci; RIL, recombinant inbred line.

Low levels of winter hardiness limit the area of commercial winter oat (*Avena* spp.) production in much of North America and Europe. Cereal winter hardiness is controlled by several quantitative traits including crown freezing tolerance, vernalization and photoperiod responses, heading date, and plant height (Fowler et al., 1999). Crown freezing tolerance is the most important winter hardiness trait (Olien, 1967), and Marshall (1965) developed a crown freezing procedure that predicted winter field survival. Selection for improved crown freezing tolerance using this protocol led to cultivars, germplasm lines, and populations with improved winter hardiness (Marshall and Kolb, 1982; Livingston et al., 1992, 2004). Freeze stress avoidance mechanisms include photoperiod, vernalization, and heading date characteristics that delay growth of sensitive reproductive tissues until warmer temperatures arrive. Plant height tends to be correlated with these traits, as plants that flower later have more time to grow taller.

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An intergenomic reciprocal translocation associated with winter field survival and crown freezing tolerance has been identified (Santos et al., 2006; Wooten et al., 2007). This work indicated chromosomes where crown freezing tolerance genes were located, but more specific chromosomal regions have yet to be identified in oat. This contrasts with other winter cereals because quantitative trait loci (QTL) or genes for freezing tolerance have been identified in diploid wheat (*Triticum monococcum* L.) (Vagujfalvi et al., 2003), bread wheat (*T. aestivum* L.) (Limin and Fowler, 2002; Toth et al., 2003; Fowler and Limin, 2004; Kobayashi et al., 2005), and barley (*Hordeum vulgare* L.) (Hayes et al., 1993; Pan et al., 1994; Francia et al., 2004). Almost all of these QTL for freezing tolerance are also linked to QTL for other winter hardiness component traits, such as vernalization response or heading date.

The 'Kanota' × 'Ogle' recombinant inbred line (RIL) population has been studied by several researchers in oat (Siripoonwiwat et al., 1996; Holland et al., 1997; Barbosa-Neto et al., 2000; Wight et al., 2003). Kanota is a facultative winter type released in the early 1920s (Salmon and Parker, 1921). It does not have the 7C-17 translocation as is typical of *A. byzantina* C. Koch winter oat (Zhou et al., 1999; Jellen and Beard 2000). Ogle is an improved spring oat cultivar released in Illinois in 1980 (Brown and Jedlinski, 1983). Ogle has poor freezing tolerance and has the 7C-17 translocation typical of *A. sativa* L. spring oat (Jellen and Beard 2000). The difference in freezing tolerance between the parents and the large quantity of molecular marker and related QTL data accumulated in previous research make this population useful for identifying QTL for crown freezing tolerance. Identification of crown freezing tolerance QTL would provide a tool for improving winter hardiness through marker-assisted selection. This approach is particularly suitable for a low heritability trait that can be measured only under certain environmental conditions. An additional benefit would be enhanced understanding of the relationships among different winter hardiness traits. The objective of this study was to identify QTL for crown freezing tolerance in the Kanota × Ogle RIL mapping population.

MATERIALS AND METHODS

Phenotypic Evaluation

Seed of 135 RILs from the cross between the cultivars Kanota and Ogle were provided by Dr. Howard Rines of the USDA-ARS in St. Paul, MN. Crown freezing tolerance data were collected on all 135 RILs, but five lines were dropped from the subsequent QTL analysis based on questions as to their legitimacy (Wight et al., 2003).

A sets within replications experimental design with four replications was utilized. In each replication, the full complement of 135 RILs plus seven entries of one parent and eight entries of the alternate parent were assigned at random to 15 sets of 10 entries each. Five plants of each of the 150 entries were grown for 5 wk in a 9 m^2 growth chamber in the Southeastern Plant Environment Laboratory at North Carolina State University. The chamber was illuminated for a 12-h photoperiod with photosynthetic photon flux density of 300 mmol m^{-2} s^{-1} with a day temperature of 13°C and night temperature of 10°C. Seeds of each entry were planted 1.5 cm deep in five adjacent 20-cm-long nursery tubes held in racks of 100 tubes. Plants were grown in Metromix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH) and lightly watered daily with a complete nutrient solution (Livingston, 1991). At approximately the five-leaf stage, plants were transferred to a hardening growth chamber for a 3-wk cold hardening treatment. The hardening chamber held a constant 2°C with a 12-h photoperiod of photosynthetic photon flux density of 300 mmol m^{-2} s^{-1}. Plants were watered with a complete nutrient solution three times per week, and watered with tap water on alternate days.

After hardening, plants were removed from the nursery tubes and soil was washed off the roots with ice water. Roots were trimmed to 0.5 cm in length and crowns were trimmed to 5 cm in length. The crowns were placed in slits in cold, slightly-moist sponges. The crowns and sponges were sprinkled with crushed ice to prevent super cooling and sealed in plastic bags. The sealed unit was placed on a steel plumbing flange to provide thermal and structural stabilization. The prepared units were then placed in a freezer at −1.5°C for 36 h to induce second phase cold hardening (Livingston, 1996). Subsequently, the freezer temperature was decreased to −5°C at a rate of −1°C per hour. The freezer was held at −5°C for 3 h and then raised to 2°C at a rate of 2°C per hour.

Within each replication the entries were assigned to 15 sets of 10 entries each. Each set was represented by five sponges. One of the five plants representing each of the 10 entries in the set was placed in each of the five sponges. Thus each sponge contained 10 plants each representing a different entry. The five sponges representing each 10-entry group were placed on five different shelves in the freezer. This modification to the Marshall (1965) protocol permitted more precise estimates of entry means by modeling the variation caused by the different sponges within each replication.

After the crowns and sponges thawed, the roots were removed from the crowns by trimming with scissors, and the crowns were planted in 50- by 30-cm plastic flats filled 5 cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory where environmental conditions were the same as those provided prehardening. After 3 wk of regrowth, recovery for each crown was visually measured on a scale of 0 to 10 (0 = dead, 10 = no freezing damage). Phenotypic estimates of crown freezing tolerance for each entry were based on a mean of 20 crowns, five crowns per replication.

The data were analyzed using the MIXED procedure of SAS (Littell et al., 1996) with the Satterthwaite option for calculating degrees of freedom. Narrow-sense heritability was estimated for the population excluding parental checks using an all random effects (entry, replication, set, and sponge) model following the method described by Holland et al. (2003, table 2.1 section 10), but adjusted for the differences in experimental design. Entries (including parents) were then considered

a fixed effect and the LSMEANS statement generated entry LSmeans (means). The DIFF option ($\alpha = 0.05$) was used to test for transgressive segregation among entry means. Procedure UNIVARIATE was used to check for a normal distribution of entry means. Correlations of entry means for crown freezing tolerance, measured in this experiment, with heading date and height of vernalized and nonvernalized entries measured in a previous study by Holland et al. (1997) were estimated with the CORR procedure.

QTL Detection

The Kanota × Ogle genetic linkage map published by Wight et al. (2003) consisted of a framework 286 markers selected and mapped from a pool of over 1000 markers genotyped in the population. This framework map of 286 markers and the corresponding genotypic data were used for map based QTL detection using Windows QTL Cartographer V.2.5 (Wang et al., 2006). Multiple interval mapping (MIM) was used to test for the presence of QTL and epistatic interactions, and to estimate their effects. The MIM QTL scan was initiated with no initial model. The likelihood ratio for the presence of a QTL was calculated every 1 cM. The Schwarz (1978) Bayesian Criterion, the default option, was used as the penalty function to prevent over-fitting the model (Zeng et al., 1999; Basten et al., 2004).

Some of the potential QTL identified in this study were in regions with limited genotyping of the framework markers, so four additional markers that mapped to nearby intervals and were genotyped on more than 80% of the RILs were inserted into the framework map (Wight et al., 2003). Mapmaker/EXP version 3.0 (Lander et al., 1987) was used to estimate the position to insert these markers on the map of Wight et al. (2003) by finding most likely position as identified by the LOD score. In LG 3+38, the marker OG41 was inserted at position 5.82. In LG 11_41+20, the marker UMN364A was inserted at position 63.47. On LG 16_23, the marker WG466 was inserted at position 85.99, and the marker CDO665C was inserted at position 90.19. Although the addition of these markers changed the map distance between adjacent markers as calculated by Mapmaker, the position of adjacent markers was not changed in the map. Quantitative trait loci detection was repeated using the augmented map and genotype data. The summary command was used to estimate the effects of the QTL and epistatic interactions.

Examination of Epistasis

Epistatic interaction between QTL was evaluated in SAS using the genotypes of the nearest markers to approximate the identified QTL. Two-way interactions between UMN13 and each of four markers, UMN433, BCD1968B, BCD1230B, or UMN5485, were tested. Only 66 of the RILs were genotyped at UMN433, therefore the genotypes at UMN433 for some of the remaining RILs were predicted based on flanking marker genotypes. For 40 RILs that were uniform for parental alleles at the flanking markers BCD1405 and OG41, which define a 20-cM region containing UMN433, the UMN433 genotype was predicted as the parental genotype. If an RIL did not have the alleles from the same parent at both flanking loci, the genotype at locus UMN433 was not predicted.

The GLM procedure was used to model the additive terms and the orthogonal epistatic interaction, and the LSMEANS statement with the PDIFF option was used to estimate and compare the means of the four different marker combination classes. Four marker combination classes are expected at two distinct loci with homozygous lines. Marker class means suggested possible complementary gene action, so duplicate or complementary gene action between each pair of loci was further evaluated using ANOVA with a coded dummy variable. If an RIL had the Kanota allele at UMN13 and the Ogle allele at the other locus (UMN433, BCD1968B, BCD1230B, or UMN5485) then it was coded 0, otherwise it was coded 1. For each pair of markers, crown freezing tolerance was modeled in the MIXED procedure using three potential models: (i) a simple linear model with the two markers (modeling simple additive gene action); (ii) a linear model with interaction (a typical test for epistasis); (iii) a model consisting of the coded variable (modeling duplicate or complementary gene action). These models were compared using the Akaike information criterion (Akaike, 1969) calculated with the MIXED procedure to identify the regression model that best fit the gene action (Rawlings et al., 1998). Finally QTL estimates were re-estimated using MIM including the epistatic interaction terms between QTL near UMN13 and each of the four other QTL near UMN433, BCD1968B, BCD1230B, or UMN5485.

RESULTS

Variance among entries for crown freezing tolerance was highly significant ($P < 0.0001$). Recombinant inbred line entry means ranged from 3.04 to 7.13, with Ogle rated 4.33 and Kanota rated 5.81 (Table 1, Fig. 1). The distribution was not normal ($P < 0.05$) and showed negative skewness. Eight lines were significantly less freezing tolerant than Ogle, and three lines were significantly more freezing tolerant than Kanota. The narrow sense heritability was 52 ± 4% on an entry mean basis (Holland et al., 2003). Crown freezing tolerance line means did not show any significant correlation with the heading date, height, or vernalization responses measured by Holland et al. (1997), indicating that the freezing tolerance in this population is not caused simply by the difference in vernalization requirement between a facultative winter and spring cultivar.

Seven QTL for crown freezing tolerance in seven different linkage groups and four epistatic interactions between QTL were identified (Table 2). Together, these main and epistatic effects accounted for 56.4% of the phenotypic variation in crown freezing tolerance (Table 2). Kanota contributed alleles that increased crown freezing tolerance at four QTL on linkage groups LG 24_26_34, LG 21+46_31+4, LG 3+38, and LG 11_41+20 which respectively accounted for 11.0, 7.5, 6.4, and 5.1% of the phenotypic variation. Ogle contributed alleles for increased crown freezing tolerance at three QTL on

Table 1. Population mean and extremes, parental phenotypes, and heritability for oat crown freezing tolerance from a recombinant inbred line population derived from a cross of 'Kanota' × 'Ogle'.

	Crown freezing tolerance
	0–10†
Population mean	4.9
Population min.	3.0***
Population max.	7.1***
Ogle	4.3
Kanota	5.8
Heritability	52 ± 4

****Significant at* $P < 0.001$ for difference between population extreme and most similar parent.

†0 = complete plant death; 10 = no freezing damage.

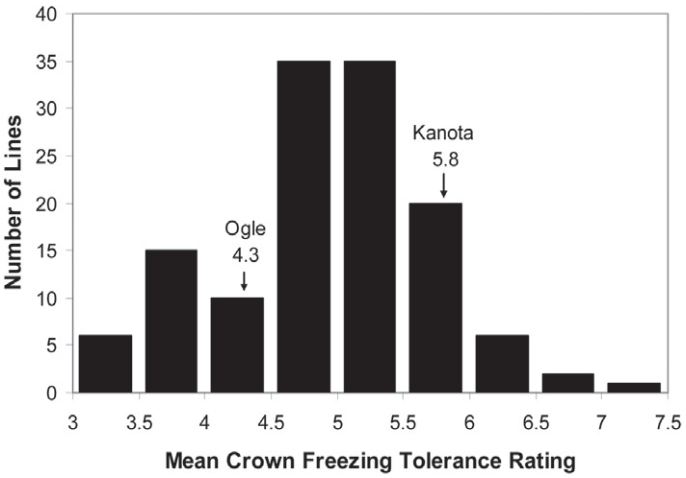

Figure 1. Frequency distribution of mean crown freezing tolerance ratings (0 = dead, 10 = no damage) of 130 recombinant inbred lines derived from a cross of 'Kanota' and 'Ogle' with parental checks.

linkage groups LG 16_23, LG 25, and LG 22_44_18 and respectively accounted for 6.5, 6.4, and 3.0% of the phenotypic variation.

One epistatic interaction was detected between the QTL on LG 3+38 (near marker UMN433) and LG16_23 (near marker UMN13) using MIM. This interaction accounted for 6.7% of the crown freezing tolerance variation. Analysis of the four marker class means indicated that the interaction followed the pattern of complementary epistasis for decreased freezing tolerance or duplicate gene epistasis for increased freezing tolerance (Table 3). The mean for RILs with the Ogle alleles at UMN433 and Kanota alleles at UMN13 was 4.37, and was significantly less ($P < 0.001$) than the other three two-locus genotype group means that ranged from 5.12 to 5.24. These latter three class means were not significantly different from each other ($P > 0.6$). Model comparison using the Akaike information criterion showed that the complementary gene action model for decreased freezing resistance best fit the genetic action of these loci (Table 4).

The occurrence of additional epistatic combinations involving the QTL near UMN13 was investigated. The interactions between three marker pairs BCD1968B with UMN13, BCD1230B with UMN13, and CDO1090C with UMN13 all followed a similar pattern to that described above for UMN433 and UMN13. The interaction terms in the additive plus orthogonal interaction model were not significant in the three combinations (confirming the MIM results), but analysis of the two-locus genotypic means showed strong evidence of duplicate or complementary epistasis (Table 3). The coded regression modeling duplicate or complementary gene action was superior for all three pairs of markers (Table 4). The coded regression model was highly significant

Table 2. Quantitative trait loci (QTL) associated with crown freezing tolerance. A population of 135 recombinant inbred lines (RILs) was scored for crown freezing tolerance using a controlled crown freezing test. Map-based QTL detection was conducted using multiple interval mapping (MIM) in QTL Cartographer V 2.5.

QTL	Linkage group†	Position cM	Nearest marker(s)	MIM LOD‡	Additive effect§	R^2%
1	*24*_26_34	41	BCD1968B	1.79	0.24	11.0
2	21+46_*31*+4	70	BCD1230B	1.31	0.22	7.5
3	*16*_*23*	111	UMN13	1.40	−0.21	6.5
4	*25*	0	waaccac273	1.24	−0.22	6.4
5	*3*+38	23	UMN433	1.17	0.19	6.4
6	11_41+*20*	61	CDO1090C	1.22	0.18	5.1
7	*22*_44_18	157	UMN5485	0.66	−0.15	3.0
5 × 3	Epistatic interaction		UMN433 × UMN13	1.17	0.19	6.7
2 × 3	Epistatic interaction		BCD1230B × UMN13	0.20¶	0.09	2.3
1 × 3	Epistatic interaction		BCD1968B × UMN13	0.16¶	0.07	0.9
6 × 3	Epistatic interaction		CDO1090C × UMN13	0.08¶	0.05	0.6

†Linkage group from Wight et al. (2003). Number in italics is location of QTL on linkage group from *O'Donoughue et al. (1995)*.

‡MIM LOD score is not equivalent to composite interval mapping LOD score due to penalty function.

§Additive effect of a 'Kanota' allele on crown freezing tolerance measured on a scale of 0 to 10.

¶Inclusion of this epistatic term is based on identification of interaction between these QTL in SAS.

($P < 0.001$) for all marker pairs, hence each of these interactions was included in the MIM model (Table 2).

Notably, epistasis was not detected in combinations between the four loci, BCD1230B, UMN433, BCD1968B, and CDO1090C, where Kanota donated alleles for increased crown freezing tolerance (Table 2). This suggested that the locus near UMN13 was involved in complementary gene interaction for freezing sensitivity with the four loci where Kanota donated alleles for freezing tolerance.

DISCUSSION

Transgressive Segregation

Three of 130 Kanota × Ogle RILs (2.3%) were transgressive segregants with increased freezing tolerance compared with 20.2% in the 'Fulghum' × 'Wintok' population examined by Santos et al. (2006). Eight Kanota × Ogle RILs (6.2%) were significantly less freezing tolerant than Ogle, while no lines in the Fulghum × Wintok population demonstrated transgressive segregation for lower freezing tolerance (Santos et al., 2006). Ogle, a spring oat, did contribute three freezing tolerance alleles to its progeny (Table 2). The inheritance of factors for improved winter hardiness from spring-type parents is a relatively common phenomenon (Amirshahi and Patterson, 1956; Murphy, 1958). The RILs that were less freezing tolerant than Ogle can be attributed to alleles exhibiting complementary epistasis for low freezing tolerance donated by Kanota and Ogle (Table 2). When an RIL contained homozygous Kanota alleles at QTL 3 (near marker UMN13) and homozygous Ogle alleles at QTL 1, 2, 5, or 6, the epistatic interaction of those loci resulted in less crown freezing tolerance than when an RIL had Ogle alleles at both loci. All of the RILs with freeze tolerance significantly lower freezing tolerance than Ogle had this combination of QTL.

Freezing Tolerance QTL

Quantitative trait loci for freezing tolerance are commonly linked with other winter hardiness component trait QTL in winter cereals (Pan et al., 1994; Galiba et al., 1995; Toth et al., 2003). Correspondence of freezing tolerance QTL identified in this study with other QTL for oat winter hardiness component traits both confirmed the significance of the freezing tolerance QTL and contributed to the understanding of the relationship between winter hardiness component traits. Most of the genomic regions identified as freezing tolerance QTL in this study were associated with vernalization response, heading date, or plant height by previous researchers (Holland et al., 1997; Siripoonwiwat et al., 1996). This supports the importance of these chromosomal regions in conferring winter hardiness.

The largest QTL effect for crown freezing tolerance in our study was near the locus BCD1968B on LG 24_26_34. Holland et al. (1997) identified this genomic region as the largest QTL affecting vernalization response in the Kanota × Ogle population (R^2 = 29%). The Kanota allele imparted a greater vernalization response for heading date than the Ogle allele in controlled environment studies. In addition, the Kanota allele increased heading date in field evaluations (Siripoonwiwat et al., 1996). In a second mapping population from a cross of Ogle by the winter cultivar TAM O-301, the same pattern of vernalization response was observed at the corresponding map location (Holland et al., 2002). A syntenous region on wheat chromosome $5A^m$ in diploid *T. monococcum* contains the vernalization gene *Vrn-A^{m}1* (Dubcovsky et al., 1998), and in hexaploid wheat this region is associated with linked vernalization and freezing tolerance genes (Galiba et al., 1995; Sutka et al., 1999). This genomic region seems to plays a key role in winter hardiness component traits throughout the *Pooideae* subfamily.

Table 3. Marker class crown freezing tolerance means for combinations of UMN13 with UMN433, BCD1968B, BCD1230B, and CDO1090C. Loci are homozygous for either parent 'Kanota' or 'Ogle'.

	UMN433†		BCD1968B		BCD1230B		CDO1090C	
UMN13	Ogle	Kanota	Ogle	Kanota	Ogle	Kanota	Ogle	Kanota
Ogle	5.24a‡	5.12a	5.12a	5.31a	5.04a	5.12a	5.04a	5.24a
Kanota	4.37b	5.17a	4.39b	5.22a	4.34b	5.02a	4.36b	5.04a

†Genotype of UMN433 marker was estimated for 40 recombinant inbred lines (RILs) uniform for flanking markers BCD1405 and OG41 that were not genotyped for UMN433. Marker UMN433 was directly genotyped in 66 RILs used in this analysis.

‡Genotype class means followed by the same letter are not significantly different at the 0.05 level.

Table 4. Akaike information criterion (AIC) for alternate models of QTL interaction. A lower AIC value indicates a better model.

Marker pair	Additive model (no interaction)	Additive plus interaction	Coded regression†
BCD1968B × UMN13	221.1	219.5	218.1
BCD1239B × UMN13	146.6	144.3	143.0
UMN433‡ × UMN13	231.7	224.1	221.9
CDO1090C × UMN13	257.9	256.0	254.5

†Coded regression models complementary gene action for decreased freezing tolerance or duplicate gene action for increased freezing tolerance

‡Genotype of UMN433 marker was estimated for 40 recombinant inbred lines (RILs) uniform for flanking markers BCD1405 and OG41 that were not genotyped for UMN433. Marker UMN433 was directly genotyped in 66 RILs used in this analysis.

A second freezing tolerance QTL was located on LG 3+38 near UMN433 in a region associated with heading date. The Kanota allele in this region increased heading date in both controlled environment (Holland et al., 1997) and field studies (Siripoonwiwat et al., 1996), which indicated the allele imparted per se lateness.

The 7C-17 intergenomic reciprocal translocation was associated with oat winter hardiness and freezing tolerance (Santos et al., 2006; Wooten et al., 2007). The QTL on linkage groups 3+38 and 24_26_34 were in the linkage groups associated with this translocation (Fox et al., 2001). The combined effect of these two QTL on freezing tolerance (R^2 = 17.6%) in this study was similar to the effect of the translocation on freezing tolerance (R^2 = 22%) reported by Santos et al. (2006), but less than the effect (R^2 = 52%) reported by Wooten et al. (2007). However, the likely breakage point for the translocation is at position 74 in LG 3+38 (Fox et al., 2001), almost 50 cM from the QTL on LG 3+38 identified in this study (Wight et al., 2003). Therefore, this QTL probably was not the genetic factor controlling crown freezing tolerance in the previous studies. While these QTL results confirm the importance of loci on chromosomes 7C and 17 in controlling oat winter hardiness traits, they do not identify the location of QTL on or near the translocation which were associated with greater freezing tolerance (Santos et al., 2006; Wooten et al., 2007)

The smallest QTL for increased freezing tolerance contributed by Kanota was on LG 11_41+20 near marker CDO1090C. This QTL follows the pattern in winter cereals whereby increased vernalization response and plant height can be associated with increased freezing tolerance and winter hardiness. The Kanota allele in this region increased plant height in vernalization and field studies (Siripoonwiwat et al., 1996; Holland et al., 1997), and the Kanota allele at a nearby QTL increased heading date vernalization response (Holland et al., 1997).

The freezing tolerance QTL located on LG 21+46_31+4 near marker BCD1230B was the second largest QTL in the model. This region was not associated with other winter hardiness traits in previous QTL studies (Siripoonwiwat et al., 1996; Holland et al., 1997). There have been a limited number of QTL studies in oat, and the previous studies with this population used a smaller number of RILs (Siripoonwiwat et al., 1996; Holland et al., 1997). The increased power conferred by the full set of 130 RILs in this study likely contributed to the discovery of this QTL. An additional analysis of the data from this study including only the 71 RILs used in previous QTL studies failed to identify the QTL near BCD1230B. It is possible that other winter hardiness QTL in the region have not been discovered. Finally, it is possible that there is a freezing tolerance QTL with no associated effects on flowering time or plant height at this location, but more evaluation of oat winter hardiness QTL would be needed to support this possible conclusion.

Kanota contributed alleles at three QTL for decreased freezing tolerance, and the QTL with the largest additive effect was located at position 0 of LG 25 (Table 2). The Kanota allele in this region decreased plant height in previous field and controlled environment vernalization experiments (Siripoonwiwat et al., 1996; Holland et al., 1997). LG 25 is the smallest linkage group with identified freezing tolerance QTL, and no corresponding portion of the Ogle × TAM O-301 map has been identified (Holland et al., 2002). Because the QTL mapped to position 0 on the linkage group, it was probably located outside the chromosomal region mapped by LG 25. This could account for the absence of a corresponding region in the Ogle × TAM O-301 population.

A minor QTL at which the Kanota allele decreased freezing tolerance was identified on LG 22_44_18 near marker UMN5485 (Table 2). Winter hardiness component traits were located in this region in both the Kanota × Ogle and Ogle × TAM O-301 populations (Holland et al., 1997; Holland et al., 2002). A locus was identified in the Kanota × Ogle population where the Kanota allele was associated with a negative vernalization response for heading date and reduced plant height (Siripoonwiwat et al., 1996, 1997). Both of these phenotypes tend to be associated with lower freezing tolerance. Holland et al. (2002) found a corresponding vernalization QTL in the Ogle × TAM O-301 population. Wight et al. (2003) identified this region of LG 22_44_18 as homoeologous to LG 24_26_34, where the largest QTL for freezing tolerance was found. Analysis of the interaction between these two possible homoeologous QTL suggested duplicate gene action (data not presented). In wheat, homoeologous genes for vernalization are found on wheat group 5 chromosomes (Snape et al., 2001). Because the QTL on LG 24_26_34 is syntenous with the group 5 chromosomes in wheat, we speculate that these two genomic regions for oat vernalization and freezing tolerance follow a similar pattern to the homoeologous winter hardiness QTL on the wheat group 5 chromosomes.

The most complex QTL identified was on LG 16_23 near UMN13. Although this QTL did not have the largest additive effect (a = −0.21) it was the most important QTL identified in the study because of epistatic effects. Previous research showed the Kanota allele in this region conferred a short, early phenotype that was not responsive to vernalization. (Siripoonwiwat et al., 1996; Holland et al., 1997).

Analysis of the QTL interactions of the UMN13 locus revealed a complex network of epistatic interactions. Almost all the reduction in freezing tolerance conferred by the Kanota allele near UMN13 resulted from epistatic interactions with QTL where Ogle alleles decrease freezing tolerance (Table 3). Conversely, the Kanota alleles for

additive increases in freezing tolerance (QTL 1, 2, 5, and 6 in Table 2) increased freezing tolerance when the UMN13 locus was homozygous for Kanota alleles. In other words, the additive effect of the QTL arose from partitioning the effect of complementary gene action into the additive terms of the genetic model. This QTL near UMN13 showed the same pattern of epistasis with all of the QTL where Kanota provided the alleles for increased freezing tolerance (Table 3). Epistasis of this form has an important impact on marker-assisted selection. Typical QTL detection studies use interval mapping or composite interval mapping to identify QTL and estimate their effects, often ignoring the epistatic effects. Using this study as an example, a breeder seeking to improve crown freezing tolerance would select for the Kanota allele at UMN433 and the Ogle allele at UMN13 (according to the additive model typically used in QTL studies) and expect an increase in freezing tolerance over that of Kanota. However the epistatic interaction would result in no increase in freezing tolerance over Kanota (Table 3). These results indicated that using QTL studies to identify potential targets for marker-assisted selection without investigating epistasis could be risky.

It was difficult to determine whether these loci exhibited duplicate gene action for increased freezing tolerance, or complementary gene action for decreased freezing tolerance because both types of epistatic interaction would produce the same pattern of phenotypes in this population. The QTL where alleles from Kanota increased freezing tolerance did not show the same pattern of duplicate gene interaction between each other. For example, there was no interaction between loci UMN433 and BCD1968B, and both of these QTL have different effects on vernalization (Holland et al., 1997). If both loci had duplicate gene action with UMN13, then we would expect duplicate gene interaction between them. Hence, we hypothesize that a gene from Kanota near UMN13 had a complementary epistatic interaction with the other QTL in a genetic or biochemical pathway where UMN13 is near the first gene in the pathway and the expression of QTL near BCD1968B, BCD1230B, UMN433, and CDO1090C depends on the gene near UMN13. Future analysis of these QTL with crown histology studies (Livingston et al., 2005) could describe how and why these genes interact. Alternatively, mapping expression of oat expressed sequence tag sequences (Bräutigam et al., 2005) homologous to known cereal winter hardiness genes may effectively characterize the function of some of the QTL identified in this study.

Unfortunately, MIM did not produce a parsimonious model for the kind of complementary epistatic interactions found in this population. Modeling duplicate or complementary gene action is difficult because the genetic effects cannot be orthogonally divided between additive and epistatic genetic effects using MIM in QTL Cartographer. The method we used in SAS with coded dummy variables modeled the genetic action, but it was not orthogonal to the QTL main effects. Although we identified complementary gene action between several QTL pairs, we had to estimate these gene effects using the orthogonal model in MIM to build a multiple locus model.

In this population we had closely linked markers and could test for potential epistatic combinations using analysis of variance. The ANOVA provided good justification for including the epistatic terms, despite the low LOD values in the MIM model (Table 2). It is reasonable to reduce the threshold for incorporating epistatic interactions between main effects QTL because there is not the same degree of multiple comparison problems as when searching for main effect QTL. Furthermore, the potential consequences of identifying a QTL for marker-assisted selection and then making a Type II error by not identifying epistatic interaction(s) are more costly than making a Type II error in identification of additive effect QTL.

It was expected that many of the freezing tolerance QTL identified in this research would be associated with vernalization response because many authors have reported tight linkage between freezing tolerance genes and vernalization genes in winter cereals (Pan et al., 1994; Storlie et al., 1998; Sutka et al., 1999; Toth et al., 2003; Kobayashi et al., 2005). It is not possible to discern linkage from pleiotropic effect in this population but the similarity between these results and those from other winter cereals supported the linkage hypothesis for most of the QTL.

The lack of a correlation between freezing tolerance and vernalization traits, as measured by Holland et al. (1997), was unexpected. The extensive epistasis between QTL may explain the lack of phenotypic correlation. Similar complementary gene action was not detected for these QTL in the vernalization experiment. Regions near UMN13 did not show any significant epistasis for any traits (Holland et al., 1997). Additionally, several genomic regions affecting freezing tolerance were not associated with QTL for vernalization traits; for example, the QTL on LG 21+46_31+4 near BCD1230B. The QTL on LG24_26_34 was the largest additive QTL for freezing tolerance and for heading date vernalization response (Holland et al., 1997) and this may have contributed to heading date vernalization response being the phenotypic trait with the closest correlation to freezing tolerance ($P = 0.07$, $r = 0.22$). Although the line mean correlations were not statistically significant, most of the freezing tolerance QTL were in genomic regions previously associated with winter hardiness component traits. However the effect of these genomic regions on other winter hardiness component traits varied. Identification of these different winter hardiness component trait QTL allowed a better description of the relationship between the winter har-

diness component traits than could have been identified by simply examining phenotypic data. This illustrates the utility of QTL analysis in dissecting the genetic architecture of oat winter hardiness component traits.

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